two conformations, an inactive conformation (R) and an activated conformation (R*), and that an equilibrium exists between these two states that markedly favors R over R* in the majority of receptors. It has been proposed that in some native receptors and in the mutants described above, there is a shift in equilibrium in the absence of agonist that allows a sufficient number of receptors to be in the active R* state to initiate signaling.

A2

On page 11, paragraph 0020, delete in its entirety and replace with the following:

The invention provides, in yet a further embodiment a compound selected from the group consisting of SEQ ID NOS:2, 4, 6, 8, 10, 12, 13, 15, 17, 21, 23, 25-27, 30, 32, 34, 36, 38, 40, 45-85, 94-111, 125-150, 160-164, 175-178 and 183-264.

A3

On page 11, paragraph 0021, delete in its entirety and replace with the following:

In yet a further embodiment, the invention provides a method for providing a therapeutic G protein coupled receptor signaling modifier peptide to a mammal which comprises administering to said mammal an expression construct which expresses a peptide according to SEQ ID NOS:2, 4, 6, 8, 10, 12, 13, 15, 17, 21, 23, 25-27, 30, 32, 34, 36, 38, 40, 45-85, 94-111, 125-150, 160-164, 175-178 and 183-264.

A4

On page 12, paragraph 0028, delete in its entirety and replace with the following:

A5

Figure 7 is a bar graph showing competitive inhibition of high affinity peptides to rhodopsin by heterotrimeric Gt.

On page 12, paragraph 0029, delete in its entirety and replace with the following:

A6

Figure 8 presents ELISA results from panning CHO cells overexpressing human thrombin receptor (PAR1) using purified MBP-C-terminal fusion proteins. MBP-G11 = xxxx (SEQ ID NO: 1) LQLNLKEYNLV (SEQ ID NO: 2); PAR-13 = VRPS (SEQ ID NO: 3) LQLNRNEYYLV (SEQ ID NO: 4); PAR-23 = LSRS (SEQ ID NO: 5) LQQKLKEYSLV (SEQ ID NO:6); PAR-33 = LSTN (SEQ ID NO: 7) LHLNLKEYNLV (SEQ ID NO: 8); PAR-34 = LPQM (SEQ ID NO: 9) QRLNVGEYNLV (SEQ ID NO: 10); PAR-45 = SRHT (SEQ ID NO: 11) LRLNGKELNLV (SEQ ID NO:12).

Table I, bridging pages 22 and 23, delete in its entirety and replace with the following:

A7

Table I. Example for Construction of a Synthetic Peptide Library.

```
                              Q  R  M  H  L  R  Q  Y  E  L  L (SEQ ID NO:13)
gaggtggt nnknnknnknnk attcgtgaaaacttaaaagattgtggtcgtttc taa ctaagtaaagc
   A           B                       C                 D      E
```

(SEQ ID NO:14) n = any nucleotide base; k = guanidine or thymidine; A = restriction enzyme site; B = linker sequence; C = oligonucleotide encoding peptide sequence; D = stop codon; E = restriction enzyme site.

On page 23, Table II, delete in its entirety and replace with the following:

A8

Table II. Gα Subunit Peptides and Corresponding DNA Constructs.

Gα Subunit	Sequence											SEQ ID NO:
Gt	I atc	K aag	E gag	N aac	L ctg	K aaa	D gac	C tgc	G ggc	L ctc	F ttc	15 16
Gi1/2	I ata	K aaa	N aat	N aat	L cta	K aaa	D gat	C tgt	G ggt	L ctc	F ttc	17 18
GRi1/2	N aac	G ggc	I atc	K aag	C tgc	L ctc	F ttc	N aac	D gac	K aag	L ctg	19 20
Gi3	I att	K aaa	N aac	N aac	L tta	K aag	E gaa	C tgt	G gga	L ctt	Y tat	21 22
Go2	I atc	A gcc	K aaa	N aac	L ctg	R cgg	G ggc	C tgt	G gga	L ctc	Y tac	23 24
Go1	I att	A gcc	N aac	N aac	L ctc	R cgg	G ggc	C tgc	G ggc	L ttg	Y tac	25 26
Gz	I ata	Q cag	N aac	N aat	L ctc	K aag	Y tac	I att	G ggc	L ctt	C tgc	27 28
G11	L ctg	Q cag	L ctg	N aac	L ctc	K aag	E gag	Y tac	N aac	L ctg	V gtc	2 29
Gq	L ctc	Q cag	L ttg	N aac	L ctg	K aag	E gag	Y tac	N aat	A gca	V gtc	30 31
Golf	Q cag	R cgg	M atg	H cac	L ctc	K aag	Q cag	Y tat	E gag	L ctc	L ttg	32 33
G14	L cta	Q cag	L cta	N aac	L cta	R agg	E gaa	F ttc	N aac	L ctt	V gtc	34 35
G15/16	L ctc	A gcc	R cgc	Y tac	L ctg	D gac	E gag	I atc	N aac	L ctg	L ctg	36 37
G12	L ctg	Q cag	E gag	N aac	L ctg	K aag	D gac	I atc	M atg	L ctg	Q cag	38 39
G13	L ctg	H cat	D gac	N aac	L ctc	K aag	Q cag	L ctt	M atg	L cta	Q cag	40 41
Gs	Q cag	R cgc	M atg	H cac	L ctt	R cgt	Q cag	Y tac	E gag	L ctg	L ctc	13 42
5' - gatccgccgccaccatggga-												-tgaa-3'

(SEQ ID NOS:43, 44)

Table III, bridging pages 24 and 25, delete in its entirety and replace with the following:

Table III. Exemplary Native G Protein Sequences for Library/Minigene Construction.*

Name	Sequence	SEQ ID NO:	Name	Sequence	SEQ ID NO:
hGt	IKENLKDCGLF	15	CryptoGba1	LQNALRDSGIL	62
hGi1/2	IKNNLKDCGLF	17	GA3_UST	LTNALKDSGIL	63
G05_DRO	IKNNLKQIGLF	45	GA1_KLU	IQQNLKKSGIL	64
GAF_DRO	LSENVSSMGLF	46	GA3_UST	LTNALKDSGIL	63
Gi-DRO	IKNNLKQIGLF	45	GA1_DIC	NLTLGEAGMIL	64
hGi3	IKNNLKECGLY	21	GA2_KLU	LENSLKDSGVL	65
hGO-1	IANNLRGCGLY	25	GA2_UST	ILTNNLRDIVL	66
hGO-2	IAKNLRGCGLY	47	Mgs-XL	QRMHLPQYELL	67
GAK_CAV	IKNNLKECGLY	21	hGs	QRMHLRQYELL	13
G0_XEN	IAYNLRGCGLY	48	hGolf	QRMHLKGYELL	68
GA3_CAEEL	IQANLQGCGLY	49	GA1_COPCO	LQLHLRECGLL	69
GA2_CAEEL	IQSNLHKSGLY	50	GA1-SOL	RRRNLFEAGLL	70
GA1_CAEEL	LSTKLKGCGLY	51	GA2_SB	RRRNLLEAGLL	71
GAK_XEN	IKSNLMECGLY	52	GA1_SB	RRRNPLEAGLL	72
GA1_CAN	VQQNLKKSGIM	53	GA1_UST	IQVNLRDCGLL	73
hGZ	IQNNLKYIGLC	27	GA4_UST	RENLKLTGLVG	74
hG15	LARYLDEINLL	26	GA1_ORYSA	DESMRRSREGT	75
GA2_SCHPO	LQHSLKEAGMF	54	GQ1_DROME	MQNALKEFNLG	76
hG12	LQENLKDIMLQ	38	GA2_DIC	TQCVMKAGLYS	77
hG13	LHDNLKQLMLQ	40	GS-SCH	LQHSLKEAGMF	54
GAL_DRO	LQRNLNALMLQ	55	GA-SAC	ENTLKDSGVLQ	56
GA2_YST	ENTLKDSGVLQ	56	GA1-CE	IISASLKMVGV	78
hG14	LQLNLREFNLV	34	GA2-CE	NENLRSAGLHE	79
hG11	LQLNLKEYNLV	2	GA3-CE	RLIRYANNIPV	80
hGQ	LQLNLKEYNAV	30	GA4-CE	LSTKLKGCGLY	51
GQ_DROME	LQSNLKEYNLV	57	GA5-CE	IAKNLKSMGLC	81
G11_XEN	LQHNLKEYNLV	58	GA6-CE	IGRNLRGTGME	82
Gq_SPOSC	IQENLRLCGLI	59	GA7-CE	IQHTMQKVGIQ	83
GA1_YST	IQQNLKKIGII	60	GA8-CE	IQKNLQKAGMM	84
GA1 NEUCR	IIQRNLKQLIL	61	GA5-DIC	LKNIFNTIINY	85

*For production of minigene constructs each nucleotide sequence should be constructed to encode the amino acids MG at the N-terminus of the peptide by using 5'-gatccgccgccaccatggga-(SEQ ID NO:43) and -tgaa-3' (SEQ ID NO:44).

On page 29, delete Table IV in its entirety and replace with the following:

Table IV. Diversity in Unpanned Gq Library.

		SEQ. ID NO.
Native	LQLNLKEYNLV	2
clone #1	LLLQLVEHTLV	86
clone #2	HRLNLLEYCLV	87
clone #3	EQWNMNTFHMI	88
clone #4	SQVKLQKGHLV	89
clone #5	LRLLL*EYNLG	90
clone #6	RRLKVNEYKLL	91
clone #7	LQLRLREHNLV	92
clone #8	HVLNSKEYNQV	93

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On page 30, Table V, delete in its entirety and replace with the following:

Table V. Selection in Panned Gα11 Library.

		SEQ ID NO.
Native	LQLNLKEYNLV	2
Round 1		
1	MKLNVSESNLV	94
2	LQTNQKEYDMD	95
3	LQLNPREDKLW	96
4	RHLDLNACNMG	97
5	LR*NDIEALLV	98
6	LVQDRQESILV	99
Round 2		
1	LQLKHKENNLM	100
2	LQVNLEEYHLV	101
3	LQFNLNDCNLV	102
4	MKLKLKEDNLV	103
5	HQLDLLEYNLG	104
6	LRLDFSEKQLV	105
Round 3		
1	LQKNLKEYNMV	106
2	LQYNLMEDYLN	107
3	LQMYLRGYNLV	108
4	LPLNPKEYSLV	109
5	MNLTLKECNLV	110
6	LQQSLIEYNLL	111

A11

On page 43, Table VI, delete in its entirety and replace with the following:

Table VI. Exemplary Sequences of C-terminal Minigene Peptides.

Peptide Name	Sequence	SEQ ID NO:
Gαi	MGIKNNLKDCGLF	112
GαiR	MGNGIKCLFNDKL	113
Gαq	MGLQLNLKEYNAV	114
Gαq**	MGLQLNLKEYNTL	115
Gα12	MGLQENLKDIMLQ	116
Gα13	MGLHDNLKQLMLQ	117

A12

Paragraph 0100, bridging pages 49 and 50 delete in its entirety and replace with the following:

A13

Construction of a biased peptide library has been described previously. Martin et al., *J. Biol. Chem.* 271:361-366, 1996; Schatz et al., *Meth. Enzymol.* 267:171-191, 1996. The vector used for library construction was pJS142 (see Figure 2). This vector had a linker sequence between the LacI and the biased undecamer peptide coding sequence, as well as restriction sites for cloning the library oligonucleotide. The oligonucleotide synthesized to encode the mutagenesis library was synthesized with 70% of the correct base and 10% of each of the other bases at each position. This mutagenesis rate leads to a biased library such that there is approximately a 50% chance that any of the 11 codons will be the appropriate amino acid and approximately a 50% chance that it will be another amino acid. In addition, a linker of four random NNK (where N denotes A, C, G or T and K denotes G or T) codons were synthesized at the 5' end of the sequence to make a total of

15 randomized codons. Using this method, a library with greater than 10^9 independent clones per microgram of vector used in the ligation was constructed based on the carboxyl terminal sequence of Gαt (IKENLKDCGLF; SEQ ID NO:15). The nucleic acid used for creating this library was:

A13

5'-GAGGTGGTNNKNNKNNKNNKattcaaggagaacctgaaggactgcggcctcttcTAACTAAGTAAAGC-3', wherein N= A/C/G/T and K= G/T; SEQ ID NO:118).

On page 50, Table VI, delete in its entirety and replace with the following:

Table VII. C-Terminal Gα Subunit Peptide Library Constructs.

A14

Gα Sub-unit	RE	Linker	Peptide Coding Region	Stop	RE	SEQ ID NO:
Gs	5-GAGGTGGT	NNKNNKNNKNNK	attcgtgaaaacttaaaagattgtggtcgtttc	TAA	CTAAGTAAAGC-3'	14
G11	5-GAGGTGGT	NNKNNKNNKNNK	ctgcagctgaacctgaaggagtacaatctggtc	TAA	CTAAGTAAAGC-3'	119
G12	5-GAGGTGGT	NNKNNKNNKNNK	ctgcaggagaacctgaaggacatcatgctgcag	TAA	CTAAGTAAAGC-3'	120
G13	5-GAGGTGGT	NNKNNKNNKNNK	ctgcatgacaacctcaagcagcttatgctacag	TAA	CTAAGTAAAGC-3'	121
G15	5-GAGGTGGT	NNKNNKNNKNNK	ctcgcccggtacctggacgagattaatctgctg	TAA	CTAAGTAAAGC-3'	122
Gz	5-GAGGTGGT	NNKNNKNNKNNK	atacagaacaatctcaagtacattggcctttgc	TAA	CTAAGTAAAGC-3'	123

On page 58, paragraph 0114, delete in its entirety and replace with the following:

The panning process is illustrated in Figure 1. For screening of the library by "panning," rhodopsin receptors prepared according to Example 5 were immobilized directly on Immulon 4 (Dynatech) microtiter wells (0.1-1 µg of protein per well) in cold 35 MM HEPES, pH 7.5, containing 0.1 mM EDTA, 50 mM KCl and 1mM dithiothreitol (HEK/DTT). After shaking for one hour at 4°C,

A15

unbound membrane fragments were washed away with HEK/DTT. The wells were blocked with 100 µl 2% BSA in HEKL (35 mM HEPES; 0.1 mM EDTA; 50 mM KCl; 0.2 M α-lactose; pH 7.5, with 1 mM DTT). For rounds 1 and 2, BSA was used for blocking; in later rounds 1% nonfat dry milk was used. For the first round of panning, about 24 wells of a 96-well plate were used. In subsequent rounds, 8 wells with receptor and 8 wells without receptor were prepared.

A15

On page 61, Table IX, delete in its entirety and replace with the following:

Table IX. Light-Activated Rhodopsin High Affinity Sequences.

Clone No.	SEQ ID NO:	Sequence
Library Sequence	124	IRENLKDCGLF
8	125	LLENLRDCGMF
9	126	IQGVLKDCGLL
10	127	ICENLKECGLF
18	128	MLENLKDCGLF
23	129	VLEDLKSCGLF
24	130	MLKNLKDCGMF
3	131	LLDNIKDCGLF
4	132	ILTKLTDCGLF
6	133	LRESLKQCGLF
11	134	IHASLRDCGLF
13	135	IRGSLKDCGLF
14	136	IFLNLKDCGLF
15/28	137	IRENLEDCGLF
16	138	IIDNLKDCGLF
17	139	MRESLKDCGLF
19	140	IRETLKDCGLL
26	141	ILADVIDCGLF
27	142	MCESLKECGLF

A16

On page 62, Table X, delete in its entirety and replace with the following:

Table X. Dark-Adapted Rhodopsin High Affinity Sequences.

A17

Clone No.	SEQ ID NO:	Sequence
Library Sequence	124	IRENLKDCGLF
2	143	IREKWKDLALF
3	144	VRDNLKNCFLF
7	145	IGEQIEDCGPF
17	146	IRNNLKRYGMF
21	147	IRENLKDLGLV
26	148	IRENFKYLGLW
33/37	149	SLEILKDWGLF
41	150	IRGTLKGWGLF

On page 62, paragraph 0118, delete in its entirety and replace with the following:

A18

The methods of Example 7 were used to screen different sources of PAR1 receptor using the Gq library. Purified PAR1, reconstituted in lipid vesicles (Example 6), membranes prepared from Sf9 insect cells expressing PAR1 (Example 2) and membranes prepared from mammalian cells overexpressing PAR1 were used. The results of the screens are presented in Tables XI, XII and XIII, respectively. The peptide used as the competitor was LQLNLKEYNLV (SEQ ID NO:2).

On page 63, Table XI, delete in its entirety and replace with the following:

Table XI. Reconstituted Purified Recombinant PAR1 Receptor; Screening Results.

Clone		SEQ ID NO:	LQLNLKEYNLV	SEQ ID NO: 2
R2-16	*SWV	151	LQFNLNDCNLV	102
R2-17	FVNC	152	LQRNKKQYNLG	160
R2-18	EVRR	153	MKLKLKEDNLV	103
R2-20	*RVQ	154	HQLDLLEYNLG	104
R2-21	RLTR	155	LQLRYKCYNLV	161
R3-37	SR*K	156	LQQSLIEYNLL	111
R3-38	MTHS	157	VHVKLKEYNLV	162
R3-44	SGPQ	158	LQLNVKEYNLV	163
R3-46	ML*N	159	LRIYLKGYNLV	164

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On page 63, Table XII, delete in its entirety and replace with the following:

Table XII. PAR1 Receptor Sf9 Insect Cell Membranes; Screening Results.

Clone		SEQ ID NO:	LQLNLKEYNLV	SEQ ID NO: 2
S1-13	S*IR	165	MKLNVSESNLV	94
S1-18	RWIV	166	LQLNLKVYNLV	175
S1-23	G*GH	167	LELNLKVYNLF	176
S2-26	RSEV	168	LQLKHKENNLM	100
S2-30	CEPG	169	LHLNMAEVSLV	177
S2-36	HQMA	170	LQVNLEEYHLV	101
S3-6	VPSP	171	LQKNLKEYNMV	106
S3-8	QMPN	172	LQMYLRGYNLV	108
S3-10	MWPS	173	LKRYLKESNLV	178
S3-15	C*VE	174	MNLTLKECNLV	110

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On page 63, Table XIII, delete in its entirety and replace with the following:

A21

Table XIII. Mammalian (CHO) Cells Overexpressing PAR1; Screening Results.

Clone		SEQ ID NO:	LQLNLKEYNLV	SEQ ID NO: 2
C4-5	PRQL	179	LQLKRGEYILV	183
C4-19	VRPS	3	LQLNRNEYYLV	3
C5-10	SRHT	11	LRLNGKELNLV	12
C5-12	FFWV	180	CSLKLKAYNLV	184
C4-16	ORDT	181	LQMNHNEYNLV	185
C7-3	NFRN	182	PQLNLNAYNLV	186
C7-10	LPQM	9	QRLNVGEYNLV	10
C7-13	LSTN	7	LHLNLKEYNLV	8
C7-14	LSRS	4	LQQKLKEYSLV	6

On page 64, Table XIV, delete in its entirety and replace with the following:

A22

Table XIV. β2-Adrenergic Receptor screened with Gs library.

Competitor	QRMHLRQYELL	SEQ ID NO 13	ELISA
AG1	QGMQLRRFKLR	187	.435
AG20	RWLHWQYRGRG	188	.431
AG19	PRPRLLRFKIP	189	.361
AG2	QGEHLRQLQLQ	190	.330
AG4	QRLRLGPDELF	191	.291
BAR1	QRIHRRPFKFF	192	.218
AG3	QRMPLRLFEFL	193	.217
BAR2	QRVHLRQDELL	194	.197
AG11	DRMHLWRFGLL	195	.192
AG9	QRMPLRQYELL	196	.190
BAR3	QWMDLRQHELL	197	.185
AG18	QRMNLGPCGLL	198	.155
BAR20	NCMKFRSCGLF	199	.079
AG13	QRLHLRGYEFL	200	.054
BAR11	HRRHIGPFALL	201	.048
BAR8	ERLHRRLFQLH	202	.047
BAR40	PCIQLGQYESF	203	.028
BAR31	QRLRLRKYRLF	204	.026

On page 65, Table XV, delete in its entirety and replace with the following:

Table XV. Rhodopsin screened with Gt library.

		SEQ ID NO:	
Competitor	IRENLKDCGLF	124	ELISA
L33	IVEILEDCGLF	205	1.007
L4	MLDNLKACGLF	206	.908
L3	ILENLKDCGLF	207	.839
L14	LRENLKDCGLL	208	.833
L38	LLDILKDCGLF	209	.823
L15	VRDILKDCGLF	210	.621
L34	ILESLNECGLF	211	.603
L17	ILQNLKDCGLF	212	.600
L7	MLDNLKDCGLF	213	.525
L10	IHDRLKDCGLF	214	.506
L20	IRGSLKDCGLF	135	.423
L6	ICENLKDCGLF	215	.342
L8	IVKNLEDCGLF	216	.257
L13	ISKNLRDCGLL	217	.187
L10	IRDNLKDCGLF	218	.162

A23

On page 66, paragraph 0120, delete in its entirety and replace with the following:

A24

Chinese hamster ovary-expressed PAR1 was screened against the Gt, G12 and G13 libraries, using the competitor peptide indicated in Table XVI below. Additional peptide analogs were identified using the G11 library and LQLNLKEYNLV (SEQ ID NO:2) as competitor and screened for high affinity binding to PAR1 receptor obtained from different sources, indicated in Table XVII, below.

On page 66, Table XVI, delete in its entirety and replace with the following:

A25

Table XVI. Peptides Identified with CHO EXPRESSED PAR1.

Gt library (IRENLKDCGLF; SEQ ID NO:124)	G12 library (LQENLKDIMLQ; SEQ ID NO:38)	G13 library (LQDNLKQLMLQ; SEQ ID NO:233)
IREFLTDCGLF 219	LQENLKEMMLQ 225	LQDNLRHLMLQ 234
IRLDLKDVSLF 220	LEENLKYRMLD 226	LQDKINHLMLQ 235
ICERLNDCGLC 221	LQEDLKGMTLQ 227	LQANRKLGMLQ 236
PRDNTKVRGLF 222	LQETMKDQSLQ 228	LIVKVKQLIWQ 237
FWGNLQDSGLF 223	PQVNLKSIMRQ 229	MRAKLNNLMLE 238
RRGNGKDCRHF 224	WQHKLSEVMLQ 230	LQDNLRHLIQ 239
	LKEHLMERMLQ 231	LQDNRNQLLF 240
	LLGMLEPLMEQ 232	

On page 67, Table XVII, delete in its entirety and replace it with the following:

Table XVII. PAR1 Binding Peptides Screened using a G11 Library (LQLNLKEYNLV; SEQ ID NO: 2)

CHO EXPRESSED	SEQ ID NO:	Recomb/Reconst	SEQ ID NO:	SF9 EXPRESSED	SEQ ID NO:
LQLNVKEYNLV	163	LQLNVKEYNLV	163	LQLNLKVYNLV	175
LQLNRKNYNLV	241	LQLRVKEYKRG	244	LQLKHKENNLM	100
LQLRYKCYNLV	161	LQLRYKCYNLV	161	LQKNLKEYNMV	106
LQLDLKESNMV	242	LQIYLKGYNLV	245	LQVNLEEYHLV	101
LQLNLKKYNRV	243	LQFNLNDCNLV	102	LFLNLKEYSLV	257
LQLRVKEYKRG	244	LQRNKKQYNLG	160	LELNLKVYNLV	258
LQRNKKQYNLG	160	LQRNKNQYNLG	254	LPLNPKEYSLV	109
LQIYLKGYNLV	245	LQQSLIEYNLL	111	LPLNLIDFSLM	259
LQFNLNDCNLV	102	LRLDFSEKQLV	105	LPRNLKEYDLG	260
LQYNLKESFVV	246	LYLDLKEYCLF	255	LRLNDIEALLV	261
LQQSLIEYNLL	111	HQLDLLEYNLG	104	LVLNRIEYNLL	262
LQRDHVEYKLF	247	VQVKLKEYNLV	251	LHLNMAEVSLV	177
LVIKPKEFNLV	248	MKLKLKEDNLV	103	MNLTLKECNLV	110
IQLNLKNYNIV	249	SAKELDQYNLG	256	MKLNVSESNLV	94
HQLDLLEYNLG	104			LKRYLKESNLV	178
MQLNLKEYNLV	250			LKRKLKESNMG	263
VQVKLKEYNLV	251			LKRKVKEYNLG	264
QLLNQYVYNLV	252				
MKLKLKEDNLV	103				
WRLSLKVYNLV	253				

A26

Paragraph 0121, bridging pages 67 and 68, delete and replace with the following:

A27

In the last round of panning, several clones were selected from the (+) receptor plates and grown up overnight in LB-Amp media. Three hundred microliters of the overnight culture was diluted in 3 mL in LB-Amp media for "ELISA lysate culture." Another 30 µL was added to an equal volume of 50% glycerol was

stored in labeled microcentrifuge tubes at -70°C. The remaining 4.5 mL was used to make DNA using a standard miniprep protocol (Qiagen Spinprep™ kits) and sequenced using a 19 base pair reverse primer which is homologous to the vector at a site 56 basepairs downstream from the TAA stop condon that terminates the random region of the library (GAAAATCTTCTCTCATCCG; SEQ ID NO:265). The DNA was stored at -20°C. The ELISA lysate culture was allowed to shake for one hour at 37°C. Expression was induced by adding 33 µL 20% arabinose (0.2% final concentration) with shaking at 37°C for 2-3 hours. The culture then was subjected to sedimentation at 4000 xg for five minutes, the pellet resuspended in 3 mL cold WTEK buffer, resedimented at 4000 xg for five minutes and the pellet resuspended in 1 mL cold TEK buffer. After transfer to 1.5 mL microcentrifuge tubes, the pellet was sedimented at 13,000 xg for two minutes and the supernatant aspirated. The cell pellet was resuspended in 1 mL lysis buffer (42 mL HE, 5 mL 50% glycerol, 3 mL 10 mg/mL BSA in HE, 750 µL 10 mg/mL lysozyme in HE and 62.5 µL 0.2 M PMSF) and incubated on ice for one hour. One hundred ten microliters 2M KCl was added to the lysis mixture and inverted to mix, then sedimented at 13,000 xg for 15 minutes at 4°C. The clear crude lysate (about 0.9 mL supernatant) was transferred to a new tube and stored at -70°C.

A27

Paragraph 0123, bridging pages 69 and 70, delete and replace with the following:

To identify peptides having even higher affinity to light-activated rhodopsin than those identified by the panning procedure described in Example 7, a high affinity peptide was included in the library incubations in rounds three and four. Peptide 8 (LLENLRDCGMF; SEQ ID NO:125) had been identified in the first screening as a peptide exhibiting binding to light-activated rhodopsin 1000-fold higher than the native sequence. Screening of the Gαt library was performed as in Example 7, except that 10 µL 100 µM (100 nM final concentration) peptide 8 was included in the wells in rounds three and four. This screen revealed several clones that both bind rhodopsin with very high affinity and stabilize it in its active form, metarhodopsin II. See Table XVIII, below. Comparing Tables IX and XVIII, it is clear that the use of peptide 8 in the screen resulted in a change at position 341 to a neutral residue. Residues L344, C347 and G348 remained stable whether peptide 8 was included in the screen or not. Use of peptide 8 resulted in a higher incidence of isoleucine at position 340 (17% with native peptide versus 71% with peptide 8) and a lower incidence of glutamine at position 342 (67% with native peptide versus 29% with peptide 8) This type of information not only contributes to the discovery of highly potent analog peptides for use as drugs or drug screening compounds, but also furthers the understanding of the structural

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A28 framework which underlies the sites of contact between Gα and receptor.

On page 71, Table XVIII, delete in its entirety and replace it with the following:

Table XVIII. Exemplary Light-Activated Rhodopsin High Affinity Sequences Identified in Screens with Addition of Peptide 8.

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Clone No.	SEQ ID NO:	Sequence
Library Sequence	124	IRENLKDCGLF
Peptide 8	125	LLENLRDCGMF
3	266	ILENLKDCGLL
7	213	MLDNLKDCGLF
8	216	IVKNLEDCGLF
10	218	IRDNLKDCGLF
13	217	ISKNLRDCGLL
17	212	ILQNLKDCGLF
19	206	MLDNLKACGLF

Paragraph 0136, bridging pages 78 and 79, delete in its entirety and replace with the following:

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cDNA encoding the last 11 amino acids of Gα subunits was synthesized (Great American Gene Company) with newly engineered 5'- and 3'- ends. The 5'- end contained a BamHI restriction enzyme site followed by the human ribosome-binding consensus sequence (5'- GCCGCCACC-3'; SEQ ID NO:267), a methionine codon (ATG) for translation initiation, and a glycine codon (GGA) to protect the ribosome binding site during translation and the nascent peptide against proteolytic degradation. A HindIII restriction enzyme site was synthesized at the 3' end immediately following the translational stop codon (TGA). Thus, the full-

length 56 bp oligonucleotide for the Gi$\alpha_{1/2}$ carboxyl terminal sequence was 5'-gatccgccgccaccatgggaatcaagaacaacctgaaggactgcggcctcttctgaa-3' (SEQ ID NO:268) and the complimentary strand was 5'-agctttcagaagaggccgcagtccttcaggttgttcttgattcccatggtggcggcg-3' (SEQ ID NO:269). See Figure 11. As a control, oligonucleotides encoding the Gαi$_{1/2}$ carboxyl terminus in random order (GαiR) with newly engineered 5'- and 3'- ends also were synthesized. The DNA was diluted in sterile ddH_2O to form a stock concentration at 100 μM. Complimentary DNA was annealed in 1X NEBuffer 3 (50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM DTT; New England Biolabs) at 85°C for 10 min then allowed to cool slowly to room temperature. The DNA then was subjected to 4% agarose gel electrophoresis and the annealed band was excised. DNA was purified from the band using a kit, according to the manufacture's protocol (GeneClean II Kit, Bio101). After digestion with each restriction enzyme, the pcDNA 3.1(-) plasmid vector was subjected to 0.8% agarose gel electrophoresis, the appropriate band cut out, and the DNA purified as above (GeneClean II Kit, Bio101). The annealed/cleaned cDNA was ligated for 1 hour at room temperature into the cut/cleaned pcDNA 3.1 plasmid vector (Invitrogen) previously cut with BamHI and HindIII. For the ligation reaction, several different ratios of insert to vector cDNA (ranging from 25 μM:25 pM to 250 pM:25 pM annealed cDNA) were plated. Following the ligation reaction, the samples were heated to 65°C for 5 min to deactivate the T4 DNA

ligase. The ligation mixture (1 µl) was electroporated into 50 µl competent cells as described in Example 7 and the cells immediately placed into 1 ml of SOC (Gibco). After 1 hour shaking at 37°C, 100 µl of the electroporated cells containing the minigene plasmid DNA was spread on LB/Amp plates and incubated at 37°C for 12-16 hours. To verify that insert was present, colonies were grown overnight in LB/Amp and their plasmid DNA purified (Qiagen SpinKit). The plasmid DNA was digested with Ncol (New England Biolabs, Inc.) for 1 hour at 37°C and subjected to 1.5% (3:1) agarose gel electrophoresis. Vector alone produced 3 bands. When the 56 bp annealed oligonucleotide insert is present, there is a new NcoI site resulting in a shift in the band pattern such that the digest pattern goes from three bands (3345 bp, 1352 bp, 735 bp) to four bands (3345 bp, 1011 bp, 735 bp, 380 bp). See Figure 12. DNA with the correct electrophoresis pattern was sequenced to confirm the appropriate sequence. This method may be used to insert any high affinity peptide to create a minigene constant.

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Paragraph 0138, bridging pages 80 and 81, delete in its entirety and replace with the following:

Human embryonic kidney (HEK) 293 cells were transfected using a standard calcium phosphate procedure according to the methods of Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harpor Laboratory Press, New York, vol. 1-3 (1989), the disclosures of which are hereby incorporated by reference. To confirm the transcription of minigene constructs in transfected

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